भारतीय मानक
Indian Standard

IS 9138 : 2020

एजोटोबेक्टर जीवाणु कल्चर — विशिष्टि

(तीसरा पुनरीक्षण)

Azotobacter Spp. Inoculants — Specification

(Third Revision)

ICS 65.080

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Soil Quality and Fertilizers Sectional Committee, FAD 07

FOREWORD

This Indian Standard (Third Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Soil Quality and Fertilizer Sectional Committee had been approved by the Food and Agriculture Division Council.

Azotobacter spp. is a bacterium, which fixes atmospheric nitrogen non-symbiotically and may be used as an inoculant for non-leguminous plants. Besides fixing nitrogen, it produces antifungal metabolites and certain vitamins and growth-promoting substances, which may increase seed germination and plant, stand and also improve the initial vigour of inoculated plants.

The number of *Azotobacter spp* cells in Indian soils is generally low and the extent of benefit is dependent on the amount of organic matter present in the soil. Products containing live *Azotobacter chroococcum* cells are being produced in India on a large scale and are being sold to farmers for application to non-leguminous plants as inoculants. Many brands of *Azotobacter* are marketed in the country and they have been found to vary in quality. Hence, there is an urgent need for Indian Standards not only to test the quality of the inoculants but also to provide the farmers with certified inoculants and to help the producers to improve the quality of their products.

The useful Agricultural Micro-organisms Sectional Committee felt the need to prescribe certain requirements like early germination and increase in dry mass of inoculated plants as these were considered to be important from the performance point of view. But the present trials suggested that the plant response to *Azotobacter was* inconsistent and, therefore, such parameters could not be utilized at present. However, at a later stage after repeated trials and advancement in the present-day research and technology, it might be possible to prescribe such requirements. In spite of all these limitations this product is being sold in the country and, therefore, the Sectional Committee felt that a workable standard was urgently needed which could be improved later. Directions for use of *Azotobacter* inoculants are given in Annex A.

This standard was first published in 1979 and first revision took place in 2002. Second revision undertaken in 2009. In this revision, Requirement aligned with *Fertilizer Control Order*, 1985. Amendment incorporated in this revision.

It is obligatory on the part of the manufacturers to employ qualified soil microbiologists or general microbiologists or agricultural graduates or graduates in biology trained in soil microbiology on their staff. Manufacturers shall also maintain a quality control laboratory capable of carrying out the tests according to this specification.

The composition of the Committee responsible for the formulation of this standard is given at Annex G.

For the purpose of deciding whether a particular requirement of this standard is complied with the final value, observed or calculated, expressing the result of a test or analysis shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard
AZOTOBACTER SPP. INOCULANTS — SPECIFICATION
(Third Revision)

1 SCOPE

This standard prescribes the requirements and methods of sampling and test for *Azotobacter spp. inoculants.*

2 REFERENCES

The following standards contain provisions, which through reference in this text, constitute provision of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standard indicated below:

IS No.	*Title*
1070 : 1992	Reagent grade water (*third revision*)
4905 : 2015	Random sampling and randomization procedures (*first revision*)
6850 : 1973	Specification for agar, microbiological grade

3 TERMINOLOGY

For the purpose of this standard, the following definitions shall apply.

3.1 ***Azotobacter spp. Inoculants* (AI)** — *Azotobacter spp. inoculants* is a product having a large population of a strain or a combination of several strains of *Azotobacter* for a particular non-leguminous plant or a group of non-leguminous plants. These AI provide biologically fixed nitrogen to such crops, protect plants against some diseases and thereby enhance crop yield.

3.2 Group of Non-leguminous Plants — Plants, which are not nodulated by Rhizobium, like cereals, millets, oilseeds, and non-leguminous vegetables.

4 REQUIREMENTS

4.1 Azotobacter spp. inoculants (AI) Shall be carrier-based, the colour depending on the colour of the carrier.

4.2 Azotobacter spp. inoculants (AI) shall contain a minimum CFU 5×10^7 viable *Azotobacter* cells/g of the carrier on dry-mass basis during the entire period of shelf-life from the date of manufacture. The number shall be counted by the plate count method as given in Annex B.

4.3 Azotobacter spp. inoculants (AI) shall have minimum 6 months expiry period from the date of manufacture.

4.4 Azotobacter spp. inoculants (AI) shall have no contamination with other microorganisms at 10^5 dilutions when counted as given in Annex B.

4.5 The *p*H of AI shall be between 6.5 and 7.5 when tested as given in Annex C.

4.6 The minimum amount of nitrogen fixed by AI shall not be less than 10 mg/g of sucrose utilized, when tested according to the method given in Annex D.

4.7 The carrier material, such as peat, lignite, peat soil, humus or similar material favoring growth to be neutralized with calcium carbonate and sterilized shall be in the form of a powder capable of passing through 150 to 212µ (72 to 100 mesh) IS sieve.

4.8 Specified mother culture be obtained from any recognized institution maintaining the mother cultures. The manufacturer may control the quality of the broth as given in Annex D.

4.9 The carrier based AI shall be friable (moist) with 30-40 percent by weight moisture content. Moisture content shall be determined as given in Annex E.

5 PACKING, MARKING AND STORAGE

5.1 Packing

Azotobacter spp. inoculants (AI) shall be packed in packaging material of low-density polyethylene-polypropylene bags thickness of which shall be 75-100 micron minimum.

5.2 Marking

Each packet shall be marked legible to give the following information:

a) Name of the product, specifically as Azotobacter inoculant;
b) Non-leguminous crop for which intended;
c) Name and address of the manufacturer;

d) Type of the carrier;
e) Batch or code number;
f) Date of manufacturer;
g) Date of expiry (agreed between the manufacturer and the purchaser subject to minimum 6 months from the date of manufacturer(s);
h) Net quantity and arte of application;
j) Storage instructions worded as 'store in a cool place away from direct sun and heat';
k) Number of *Azotobacter* cells/g of carrier; and
m) Any other information required under the *Legal Metrology (Packaged Commodities) Rules*, 2011.

5.2.1 Items (b), (f) and (g) above shall be printed on a coloured ink background.

5.2.2 Directions for the use of AI shall be printed briefly on the packet as given in Annex A.

A separate pamphlet may preferably be given with it.

5.2.3 *BIS Certification Marking*

The product(s) conforming to the requirements of this standard may be certified as per the conformity assessment schemes under the provisions of the *Bureau of Indian Standards Act*, 2016 and the Rules and Regulations framed thereunder, and the products may be marked with the Standard Mark.

5.3 Storage

Azotobacter spp. inoculants (AI) shall be stored by the manufacturer in a cool and dry place away from direct sun or heat preferably at a temperature of 15 °C to 30 °C. It shall also be the duty of the manufacturer to instruct the retailers and, in turn, the users about the precautions to be taken during storage.

6 SAMPLING

The method of drawing representative samples of AI from different batches and the criteria for conformity shall be as given in Annex F.

7 TESTS

7.1 Tests shall be carried out by the methods prescribed in **4**.

7.2 Unless otherwise specified, quality reagents, chemicals and distilled water (*see* IS 1070) shall be employed in tests.

NOTE — 'Pure Chemicals' shall mean chemicals that do not contain impurities which affect the results of test analysis.

ANNEX A

(*Foreword, and Clause* 5.2.2)

DIRECTIONS FOR USE

A-1 Use only for the non-leguminous crops and before the expiry date.

A-2 This is not a chemical fertilizer, hence do not mix it or the inoculated planting material with chemical fertilizers or pesticides.

A-3 Use for the crops specified on the packet.

A-4 Do not expose to direct sunlight or heat.

ANNEX B

(*Clause* 4.2 *and* 4.4)

TOTAL PLATE COUNT

B-1 APPARATUS

B-1.1 Pipettes, graduated, 1 ml and 10 ml.

B-1.2 Dilution Bottles or Flasks

B-1.3 Petri Dishes, clear, uniform, flat-bottomed.

B-1.4 Hot-Air Oven

Capable of giving uniform and adequate temperature, equipped with a thermometer, calibrated to read up to 250 °C and with vents suitably located to assure prompt and uniform heating.

B-1.5 Autoclave

B-1.6 Incubator

B-1.7 Hand Tally or Mechanical Counting Device

B-1.8 *p*H Meter

B-2 REAGENTS

B-2.1 Medium

Use a plating medium of the following composition:

Agar (*see* IS 6850)	20.0 g
Sucrose ($C_{12}H_{22}O_{11}$)	20.0 g
Ferrous sulphate ($FeSO_4.7H_2O$)	0.1 g
Dibasic potassium phosphate (K_2HPO_4)	1.0 g
Magnesium sulphate ($MgSO_4.7H_2O$)	0.5 g
Sodium chloride (NaCl)	0.5 g
Calcium carbonate($CaCO_3$)	2.0 g
Sodium molybdate (Na_2MoO_4)	0.005 g
Distilled water	1 000 ml
*p*H	6.8 to 7.2

B-2.1.1 Use nutrient agar (beef extract 3.0 g, tryptone 5.0 g, agar 15.0 g and distilled water 1 000 ml, *p*H 7.0) for total count of bacteria. The extent of contamination can be assessed by difference between the total bacterial count and *Azotobacter spp.* count.

B-2.2 Sterilizing Procedure

B-2.2.1 Sterilize the sampling and plating equipment with dry heat in a hot air oven at not less than 160 °C for not less than 3 h.

B-2.2.2 Sterilize the media by autoclaving at 121.6 °C for 15 min. To permit passage of steam into and from closed containers when autoclaved, keep stoppers slightly loosened. Air from within the chamber of the sterilizer should be ejected allowing steam pressure to rise.

B-3 PREPARATION OF PLATING MEDIUM AND POURING

B-3.1 Prepare growth medium in accordance with the composition indicated in B-2.1.

B-3.2 Melt the required amount of medium in boiling water or by exposure to flowing steam in a partially closed container but avoid prolonged exposure to unnecessarily high temperature during and after melting. Do not melt more medium than that will be used within 3 h. Re-sterilization of the medium may cause partial precipitation of ingredients.

B-3.3 When boiling time is less than 30 min, promptly cool the molten medium to about 45 °C, and store, until used, in a water-both or incubator at 46 ± 1 °C. Introduce 15 to 20 ml of liquefied medium at 44 °C to 45 °C into each plate. Gently lift the cover of the dish, only enough to insert the pipette or to pour in the medium. Sterilize the lips of the medium containers by exposure to flame:

a) Immediately before pouring,
b) Periodically during pouring operations,
c) When pouring is complete for each batch of plates, and
d) If portions of molten medium remain in containers and are to be used without subsequent sterilization for pouring additional plate.

As each plate is poured, thoroughly mix the medium in the container.

B-3.4 By rotating and tilting the dish and without splashing the mixture over edge, spread the mixture evenly over the bottom of the plate. Provide conditions so that the mixture solidifies with reasonable promptness before removing the plates from level surface.

B-3.5 Add 30 g of AI to 270 ml of sterile distilled water and shake for 10 min on a reciprocal shaker. Make serial dilutions up to 10^9. Take 0.1 ml or suitable aliquots of 10^6 to 10^9 dilutions using sterile pipettes and deliver to petri dishes containing set medium as given in **B-2.1** and spread it uniformly with a sterilized spreader. Invert the plates and promptly place them in the incubator.

B-4 INCUBATION

B-4.1 Label the plates and incubate at 28 ± 1 °C for 4 to 6 days.

B-4.2 Colony Counting Aids

Count the colonies, with the aid of magnifying lens under uniform and properly controlled artificial illumination. Use the colony counter equipped with a grid plate rules in centimeter square. Record the total number of colonies with the hand tally. Avoid mistaking particles of undissolved medium or precipitated matter in plates for pinpoint colonies. To distinguish colonies from dirt, specks and other foreign matter, examine doubtful objects carefully.

B-4.3 *Azotobacter spp.* colonies are gummy, raised with or without striations, viscous and often sticky. The pigmentation varies from very light brown to black. Count the colony number and observe the cyst formation as given below and calculate number per gram of the carrier material.

B-4.3.1 Grow the vegetative cells at 30 °C on Burk's agar medium comprising sucrose 20 g; dipotassium hydrogen phosphate 0.64 g; dihydrogen potassium phosphate 0.20 g; sodium chloride 0.20 g; calcium sulphate 0.05 g; sodium molybdate 0.001 g; ferrous sulphate 0.003 g; agar 20 g and distilled water 1 000 ml. Look for vegetative cells after 18 to 24 h either by simple staining method or through a phase contrast microscope.

B-4.3.2 for cyst formation, grow the cyst cells on Burk's agar medium as given above with 0.2 percent *n*-butanol in place of the carbon source. Look for cyst formation after 4 to 5 days incubation.

B-5 STAINING AND OBSERVATION UNDER SIMPLE MICROSCOPE

Apparatus

a) Staining tray;
b) Staining rack;
c) Slide holder; and
d) Disposable gloves.

B-6 REAGENTS

a) Crystal violet 1 percent solution (primary stain); and
b) Copper sulfate 20 percent (decolorizer agent).

B-7 PROCEDURE

a) Prepare a smear from a 12 to 18 h culture with serum protein (to provide a proteinaceous background for contrast).
b) Allow the smear to air dry. DO NOT HEAT FIX (to avoid destroying or distorting the capsule or causing shrinkage).
c) Cover the slide with 1percent crystal violet for 2 min.
d) Rinse gently with a 20 percent solution of copper sulfate.
e) Air dry the slide. DO NOT BLOT. (Blotting will remove the un-heat-fixed bacteria from the slide and/or cause disruption of the capsule.)
f) Examine the slide under an oil immersion lens. Bacterial cells and the proteinaceous background will appear purplish while the capsules will appear transparent.

   Cysts appeared round, having a discernible outer wall, a much thicker faint blue inner wall (intine) with a circular central body that stained deep purple. Vegetative cells stained uniformly deep purple.

OBSERVATION UNDER PHASE CONTRAST MICROSCOPE

Under the phase contrast microscope the center of the cyst appears to be dense and often contains refractile granules. The cyst wall appears as a discrete, non-refractive halo encompassing the body of the cyst. Vegetative cells appear uniformly dense, many with refractive granules.

ANNEX C

(*Clause* 4.5)

DETERMINATION OF *p*H

C-1 PROCEDURE

Make 25 g of AI into a suspension in 50 ml of distilled water and shake on a rotary shaker for 2 h. Filter through Whatman No. 1 or equivalent filter paper under vacuum using a Buchner funnel. Determine *p*H of the filtrate by a *p*H meter.

ANNEX D

(*Clauses* 4.6 *to* 4.8)

TEST FOR NITROGEN FIXATION IN PURE CULTURE

D-1 PURE CULTURE MEDIUM

Prepare medium as given in **B-2.1** excluding agar.

D-2 PROCEDURE

D-2.1 Select one Azotobacter colony, of the type that has been counted as *Azotobacter spp.* in **B-4.3**, grow in slant culture on the medium in **B-2.1**. Use this pure culture for inoculating the broth for nitrogen fixation. For this purpose, take 50 ml aliquots of broth in 250 ml conical flasks for inoculation. Inoculate and incubate for 7 days on a rotary shaker at 29 ± 1 °C. Test the contents of the flasks for purity by streaking on fresh medium. Concentrate the contents over a water bath (50 °C to 0 °C) or in an oven at 90 °C or under vacuum at 60 °C to dryness. Collect the dried culture and take it as a sample. The contents of the flasks in inoculated control series (flasks inoculated and kept in deep freeze to inhibit growth) should be prepared in a similar manner to serve as control.

D-2.2 Determination by Kjeldahl Method

D-2.2.1 *Reagents*

D-2.2.1.1 *Sulphuric acid,* 93 to 98 percent N-free.

D-2.2.1.2 *Mercuric oxide, N-free.*

D-2.2.1.3 *Potassium sulphate (or anhydrous sodium sulphate)*

D-2.2.1.4 *Sulphide or thiosulphate solution* — Dissolve 40 g commercial potassium sulphide in 1 litre of water (or solution of 40 g sodium sulphide or 80 g sodium thiosulphate in 1 litre may be used).

D-2.2.1.5 *Zinc granules*, reagent grade.

D-2.2.1.6 *Sodium hydroxide solution*, *N-free* — Dissolve about 450 g solid sodium hydroxide in water, cool and dilute to 1 litre (specific gravity of the solution should be at least 1.36).

D-2.2.1.7 *Hydrochloric or sulphuric acid standard solution* — Standard solution 0.5 N or 0.1 N in case of amount of nitrogen is small.

D-2.2.1.8 *Indicators*

a) Methyl red indicator — Dissolve 1 g of methyl red in 200 ml of ethanol.
b) Mixed indicator — Prepare mixed indicator by dissolving 0.8 g of methyl red and 0.2 g of methylene blue in 500 ml of ethanol.

D-2.2.1.9 *Boric acid*, 4 percent solution.

NOTE — Ration of salt to acid (*w/v*) should be about 1 : 1 at the end of the digestion for proper temperature control. Digestion may be incomplete at a lower ratio, and nitrogen may be lost at higher ratio. Each gram of fat consumes 10 ml of sulphuric acid and each gram of carbohydrate 1 ml of sulphuric acid during digestion.

D-2.2.2 *Apparatus*

D-2.2.2.1 *For digestion* — Use Kjeldahl's flasks of hard, moderately thick, well-annealed glass with total capacity approximately 500 to 800 ml. Conduct digestion over heating device adjusted to bring 250 ml of water 25 °C to rolling boil in about 5 min. To test the heaters, preheat for 10 min in the case of gas burners, and for 30 min in the case of electric heaters. Add 3 to 4 boiling chips to prevent superheating.

D-2.2.2.2 *For distillation* — Use 500 to 800 ml Kheldahi's flask fitted with rubber stopper through which passes the lower end of an efficient scrubber bulb or trap to prevent mechanical carryover of sodium hydroxide during distillation. Connect the upper end of the bulb tube to a condenser by rubber tubing. Trap the outlet of the condenser in such a way as to ensure absorption of ammonia distilled over with the receiver.

D-2.2.3 *Procedure*

D-2.2.3.1 Place 0.25 g of the sample (**D-2.1**) in the digestion flask. Add 0.7 g mercuric oxide, 15 g powdered potassium sulphate or anhydrous sodium sulphate and 25 ml of sulphuric acid. Place flask in inclined position and heat gently until frothing ceases (if necessary add small amount of paraffin to reduce frothing); boil briskly until solution becomes clear and then for 2 h. Cool, add about 200 ml of water, cool to room temperature, add 25 ml of water, cool to room temperature, add 25 ml of the sulphide or thiosulphate solution and mix to precipitate mercury. Add few zinc granules to prevent bumping.

D-2.2.3.2 Tilt the flask and add 85 ml of sodium hydroxide solution without agitation. Immediately connect the flask to distillation bulb on condenser and with tip of the condenser immersed in 50 ml boric acid solution containing 5-7 drops of indicator, rotate the receiver flask or mix contents thoroughly. Heat until all ammonia had distilled (collect about 150 ml distillate). Remove receiver, wash tip of condenser and titrate the distillate with standard acid (**D-2.2.1.7**). Correct for blank determination on reagents.

NOTE — Check the ammonia recording periodically using inorganic nitrogen control, for example, ammonium sulphate.

D-2.2.4 *Calculation*

$$\text{Nitrogen content, percent by mass, g} = \frac{T.V. \times N \times 1.4}{m}$$

where

T.V. = titration value (ml of standard acid),

N = normality of standard acid, and

M = mass of sample taken.

D-2.3 Determination of Sucrose Consumed

D-2.3.1 *Sample Preparation*

Take 1 g of accurately weighed prepared sample of AI (**D-2.1**) into 250 ml volumetric flask and dilute with about 150 ml of water. Mix thoroughly the contents of the flask and make the volume to 250 ml with water. Centrifuge the mixture for 15 min at 10 000 rpm. Decant the supernatant carefully and estimate the total reducing sugar after inversion (**D-2.3.2**).

D-2.3.2 *Method of Inversion*

To 100 ml of the supernatant (**D-2.3.1**) add 1 ml of concentrated hydrochloric acid and heat the solution to near boiling. Keep aside overnight. Normalize this solution with sodium carbonate and determine the total reducing sugars.

D-2.3.3 *Determination of Reducing Sugars*

Using separate pipettes, take accurately 5 ml each of solution A (**D-2.3.4.1**) and solution B (**D-2.3.4.2**) in porcelain dish. Add about 12 ml of AI solution from a burette and heat to boiling over an asbestos gauze. Add 1 ml of methylene blue indicator and while keeping the solution boiling complete the titration within 3 min, the end point being indicated by change of colour from blue to red. Record the volume (*H*) in ml of AI solution required for titration. Follow the same steps for control. The difference between the amount of total reducing sugars in the control and inoculated expressed in terms of sucrose gives the actual amount of sucrose consumed by the organism. Calculate the amount of nitrogen fixed per gram of sucrose consumed.

D-2.3.4 *Reagents*

D-2.3.4.1 *Copper sulphate solution (Solution A)* — Dissolve 34.639 g of copper sulphate crystals ($CuSo_4.5H_2O$) in water, dilute to 500 ml and filter through glass wool or filter paper. Standardize the solution as per **D-2.3.4.6**.

D-2.3.4.2 *Potassium sodium tartrate (Rochelle salt) solution (Solution B)* — Dissolve 173 g of potassium sodium tartrate and 50 g of sodium hydroxide in water, and dilute to 500 ml. Let the solution stand for a day and filter.

D-2.3.4.3 *Hydrochloric acid,* sp gr 1.18 at 20 °C (approximately 12 N).

D-2.3.4.4 *Standard invert sugar solution* — Weigh accurately 0.95 g of sucrose and dissolve it in 500 ml of water. Add 32 ml of concentrated hydrochloric acid, boil gently for 30 min and keep aside for 24 h. Neutralize with sodium carbonate and make the final volume to 1 000 ml; 50 ml of this solution contains 0.05 g of invert sugar.

D-2.3.4.5 *Methylene blue indicator,* 0.2 percent in water.

D-2.3.4.6 *Standardization of copper sulphate solution* — Using separate pipettes, pipette accurately 5 ml of solution A (**D-2.3.4.1**) and 5 ml of solution B (**D-2.3.4.2**) into a conical flask of 250 ml capacity. Heat this mixture to boiling on an asbestos gauze and add standard invert sugar solution (**D-2.3.4.4**) from a burette, about 1 ml less than the expected volume which will reduce the Fehling solution completely (about 48 ml). Add 1 ml of methylene blue indicator while keeping the solution boiling. Complete the titration within 3 min. The end point being indicated by change of colour from blue to red. From the volume of invert sugar solution used, calculate the strength(s) of the copper sulphate solution by multiplying the titre value by 0.001 (g/ml of the standard invert sugar solution). This would give the quantity of invert sugar required to reduce the copper in 5 ml copper sulphate solution.

D-2.3.5 *Calculation*

$$\text{Total reducing sugars, percent by mass} = \frac{250 \times 100 \times S}{H \times M}$$

where

S = strength of copper sulphate solution (g of invert sugar),

H = volume in ml of AI solution required for titration, and

M = mass in g of AI taken for the test.

Sucrose consumed, percent by mass = (Total reducing sugar, percent by mass in control – Total reducing sugar, percent by mass in inoculant) × 0.95

Nitrogen fixed, mg per gram of sucrose consumed =

$$\frac{\textbf{\textit{Nitrogen content, percent by mass}}\ (\textbf{D-2.2.4})}{\textbf{\textit{Sucrose consumed, percent by mass}}\ (\textbf{D-2.3.5})}$$

ANNEX E

(*Clause* 4.9)

TEST FOR MOISTURE

E-1 PROCEDURE

Take a known quantity (25 g approximately) of a carrier based biofertilizer in a pre-weighed can with lid or a suitable container. Weigh and note the initial weight. Dry at 105 °C in an oven. Cool in a desiccator and weigh the entire contents on a weighing balance. Repeat the process (that is, dry again in oven, cool in a desiccator and weigh) at least three times or till the constant weight is obtained. Record the loss in weight.

E-2 CALCULATION

Moisture content in percent =

$$\frac{(\textbf{\textit{Loss in weight on drying}}) \times \textbf{100}}{\textbf{\textit{Weight of the oven dry sample}}}$$

ANNEX F

(*Clause* 6)

SAMPLING OF *AZOTOBACTER SPP.* INOCULANTS

F-1 GENERAL REQUIREMENTS

F-1.0 In drawing, preparing and handling the samples, the following precautions and directions shall be observed.

F-1.1 Sampling shall be carried out by a trained and experienced person as it is essential that the sample should be representative of the lot to be examined.

F-1.2 Since the samples are also required for microbiological analysis, utmost precautions are necessary to avoid extraneous contamination while drawing and handling the samples and to preserve them in their original condition till they are ready for examination in the laboratory.

F-1.2.1 No preservative or bactericidal or fungicidal agent shall be added to samples required for microbiological analysis.

F-1.3 Intact packets should be drawn and sent to the laboratory. This will prevent possible contamination of the samples during handling and also help in revealing the true condition of the material.

F-1.4 Samples shall be drawn in a protected place not exposed to damp air, bright light, dust or soot, and transferred to sterile containers.

F-1.5 The sampling appliances and sample containers shall be clean and sterile.

F-1.6 All precautions shall be taken to protect the samples, the material being sampled, the sampling instruments and the sample container against adventitious contamination at the time of drawing samples, opening containers and transferring the samples.

F-2 SAMPLING IMPLEMENT

F-2.1 A suitable scoop made of stainless steel may be used for drawing samples.

F-2.2 The sampling equipment shall be perfectly clean and sterile. It shall be properly sterilized by heating in a hot-air oven at 160 °C for not less than 3 h or by autoclaving for not less than 15 min at 121.6 °C and held in suitable containers to prevent recontamination.

F-3 SCALE OF SAMPLING

F-3.1 Lot

AI packets in a single consignment having material from the same type of carrier and belonging to the same batch of manufacture shall constitute a lot.

F-3.2 For ascertaining conformity of the material to the requirements of specification, samples shall be tested from each lot separately.

F-3.3 The number of packets to be selected from a lot shall depend on the size of the lot and shall be according to Table 1.

Table 1 Scale of Sampling

(*Clause* F-3.3)

Number of Packets in the Lot	**Sample Size (for Requirement)**	
	Other than 4.5	of 4.5
(1)	(2)	(3)
Up to 100	6	1
101 to 1000	9	2
1001 to 10 000	12	3
10 001 and above	15	4

F-3.3.1 These packets shall be selected at random and in order to ensure the randomness of selection procedures given in IS 4905 may be followed.

F-4 NUMBER OF TESTS AND CRITERIA FOR CONFORMITY

F-4.1 The packets selected from the lot according to col 1 and 2 of Table 1 shall be divided into three equal groups. A test sample shall be prepared from each of the packets belonging to the first group to be examined for the requirements given in **4.1, 4.2** and **4.7**. A test sample shall be prepared from each of the packets belonging to the second group to be tested or *p*H (*see* **4.5**). A test sample shall be prepared from each of the packets belonging to the third group to be tested for carrier material (*see* **4.7**).

F-4.1.1 The lost shall be considered as satisfying the requirements given in **4.1, 4.2, 4.4** and **4.7**, if all the samples belonging to various groups meet the corresponding requirements.

F-4.2 The lot having been found satisfactory according to **F-4.1** shall be further tested. The number of packets given in col 3 of Table 1 shall be taken afresh from the lot and a test sample prepared for each of the packets to be tested for nitrogen fixation (*see* **4.6**).

F-4.2.1 The lot shall be deemed to have met these requirements if none of the samples so tested fails.

F-4.3 The lot shall be declared as conforming to the requirements as specified if **F-4.1** and **F-4.2** are satisfied.

ANNEX G

(*Foreword*)

COMMITTEE COMPOSITION

Soil Quality and Fertilizers Sectional committee, FAD 07

Organization	*Representative(s)*
Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya	Dr A. K. Singh (***Chairman***)
Bidhan Chandra Krishi Vishwavidyalaya, West Bengal	Prof B. Mandal Prof G. C. Hazra (*Alternate*)
CCS Haryana Agricultural University, Hissar	Head (Soil Science) Head (Microbiology) (*Alternate*)
Central Fertilizers Quality Control & Training Institute, Faridabad	Shri Shyam Babu Director
Chambal Fertilizers & Chemicals Ltd, New Delhi	Dr Naresh Prasad Dr A. K. Baranwal (*Alternate*)
Consumer Guidance Society of India, Mumbai	Dr Sitaram Dixit
Fertilizer Association of India, New Delhi	Dr. R. K. Tewatia
G B Pant University of Agri and Technology, Pantnagar	Dr Anil K. Sharma Professor
Indian Micro Fertilizers Manufacturers Association, Pune	Shri Mahesh G. Shettry
Indian Agricultural Research Institute, New Delhi	Head (Soil Science) Head (Microbiology) (*Alternate*)
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